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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

STUDIES OF COCCIDIOIDES IMMITIS RIXFORD ET GILCHRIST

by

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VII. Immuno-Allergic Reactions in Experimental Infection of the Guinea Pig

Continuing our investigations of Coccidioides immitis (1,2,3,4,5,6,7,8), we present on this occasion a study of manifestations of allergy and immunity in experimental infection of the guinea pig. We chose that animal as being sensitive to inoculation with the fungus in question and easy to handle and to obtain.

This work was planned and carried out by Drs. Negroni and Bonfiglioli, with Dr. Vívoli in charge of the histopathological study.

Work plan. We proposed to investigate the following matters: 1) at what moment of the infection cutaneous sensitivization to the coccidioidin appears; 2) what is the reaction of infected and allergic guinea pigs (sensitive to coccidioidin), when exposed to a second inoculation with a) material from a live culture and b) the same material after its vitality has been destroyed by heating; and 3) the appearance and course of circulating antibodies.

Methods and techniques. As shown in the accompanying tables, we used 5 groups of white guinea pigs weighing 350-400 grams, which we designated with the first 5 capital letters of the alphabet.

Groups A, B, and C, consisting of 15 guinea pigs each, were inoculated via the routes indicated with an infecting

dose of 10 mg (moist weight) of the product obtained by prolonged trituration in a sterile mortar of cultures of C. immitis, Argentine strain Nº 695, after incubation for 20 days at 28° C in agar broth.

Five guinea pigs in each group were subjected to subcutaneous vaccination with a suspension of spores, in progressively increasing concentration, in a physiological solution with 0.3% carbolic acid added, heated in a water bath 30 minutes at 65° C. Sterility controls made it possible for us to verify the fact that this temperature destroys the vitality of the "entospores" of the cultures. Four series of this vaccine were prepared, of which the most concentrated corresponded in opacity to tube Nº 3 of McFarland's scale and each of the others was prepared by dilution at 1/10. Each guinea pig was given 3 weekly injections of 0.5 ml during one month beginning nine days after the infecting inoculation.

The guinea pigs of group D were subjected to vaccination without previous infecting inoculation.

The study of cutaneous allergy was made by intradermic injection of polyvalent coccidioidin prepared from Argentine and US strains of C. immitis according to the technique recommended by C.E. Smith and "standardized" with the coccidioidin type (batch 29-31) kindly supplied by him.

It was decided to use the testicle in evaluating the reactions to a second inoculation because that organ, as has been demonstrated by E.R. Long (9), reacts intensely to tubercular infection, to which coccidioidomicotic infection has much similarity, and also with the object of getting uniformity in the literature. The material used in reinoculations consisted in a suspension of "entospores" in physiological solution, the opacity of which corresponded to that of Nº 3 of McFarland's scale. This suspension was divided into two fractions: a) heated in a water bath at 65° C for 30 minutes to destroy its vitality, and b) unheated. The guinea pigs were inoculated with 0.2 ml and were killed after 18-20 hours and after 5 days, with the object of observing tissue reactions that might afford us an index of allergy and immunity.

Finally, group E received only inoculation via the testicle with live and dead material. Groups D and E, then, were taken as controls, the former for the variant introduced into our experiments and the latter to appraise the reaction to testicular inoculation with "entospores" in an organism previously uninfected.

For the reactions of fixation of the complement we usod two distinct antigens. The first consisted in an emulsion of the lipides extracted from the mycelium and the second in coccidioidin, both conveniently diluted. In view of the negative

results obtained with the first, we continued our studies using coccidioidin exclusively for these reactions. This product was also used in the precipitin reactions, and for agglutination reactions we used a suspension of "entospores" whose vitality had been destroyed by heating.

Results. The intradermal reaction with pure coccidioidin gave positive results ++ at the end of one week of infection and +0± with coccidioidin diluted to 1/10. These results were accentuated in intensity in tests made a week later.

Serological tests made on four guinea pigs that were infected and four others that were infected and vaccinated, all of group A, at the end of a month gave negative results. The same results were obtained with two guinea pigs of group B at the end of 20 days after infection and two of group C after 17 days.

Serological reactions repeated at the end of three months after infection with three guinea pigs from group A and one from group B showed intensely positive fixation of the complement, but negative precipitin and agglutination reactions.

In guinea pigs inoculated with culture material in other experiments nine months to a year before and still surviving, we got intensely positive results in the complement fixation reaction from three of ten animals. The agglutination and precipitin reactions were always negative.

The results of the tissue reactions are assembled in the accompanying tables, which also contain notes of the modifications that the parasite undergoes in microscopic appearance, number, and size.

[Translator's note: The tables in the source cover a two-page spread and cannot be readily reproduced here. For convenience, all the material contained in the tables is given at this point in paragraph form.]

Group A. Guinea Pigs Infected Subcutaneously and Reinoculated Via the Testicle

Guinea pig A765. Reinoculated with dead material. Killed after 18-20 hours. Not vaccinated; reinoculated after 23 days. Macroscopic lesions: testicle: slight congestion. Histological lesions: congestion and monocytic reaction.

A795. Reinoculated with dead material. Killed after 5 days. Not vaccinated; reinoculated after 23 days. Macroscopic lesions: testicle: slight hemorrhage; granulations in the lung, spleen; hypertrophy of ganglion. Histological lesions: testicle without lesions. Pseudotuberculous granuloma with parasites in the spleen.

A789. Reinoculated with live material. Killed after

18-20 hours. Not vaccinated; reinoculated after 23 days. Macroscopic lesions: testicle: slight congestion. Histological lesions: hemorrhagic focus and nodular monocytic reaction without parasites.

A732. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated after 23 days. Macroscopic lesions: testicle of larger size, ganglion hypertrophied, granulations in the lungs. Histological lesions: testicle: nodular productive lesions and productive-exudative lesions. Tuberculoid granuloma with epithelial cells. A few parasites, without endospores and with radiate formations.

F28. Reinoculated with dead material. Killed after 18-20 hours. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle of larger size and congested. Histological lesions: Interstitial, discrete, non-speccific orchiepididymitis.

M8. Reinoculated with dead material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle of larger size and congested; granulations in the spleen. Histological lesions: nodular productive lesions and exudative lesions. Dropsical degeneration of the cells of the covering of the seminiferous tubules.

F26. Reinoculated with live material. Killed after 18-20 hours. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: orchiepididymitis with non-specific interstitial orchitis without parasites.

M7. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: nodular lesion with productive predominance. Very rare parasites, small, without endospores and with radiate formations.

F27. Reinoculated with dead material. Killed after 18-20 hours. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: Testicle enlarged in size. Histological lesions: exudative lesions. In the spleen, exudative productive lesions with a few parasites (cystic forms).

M6. Reinoculated with dead material. Killed after 5 days. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle unchanged. Histological lesions: exudative lesions with abundant eosinophiles; dropsical degeneration of cells of the seminiferous tubules. Discrete interstitial infiltration.

F25. Reinoculated with live material. Killed after 18-20 hours. Vaccinated and reinoculated at the end of 45 days. Macroscopic lesions: testicle enlarged in size and congested;

nodular lesions in liver and spleen. Histological lesions: interstitial non-specific orchiepididymitis without parasites.

M5. Reinoculated with live material. Killed after 5 days. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested; hypertrophy of ganglion. Histological lesions: lesions with productive predominance. Rare parasites, of medium size and with endospores.

Group B. Guinea Pigs Infected Intramuscularly and Reinoculated Via the Testicle

A728. Reinoculated with dead material. Killed after 18-20 hours. Not vaccinated; reinoculated after 23 days. Macroscopic lesions: testicle congested. Histological lesions: vasodilatation, monocytic inflitration.

A712. Reinoculated with dead material. Killed after 5 days. Not vaccinated; reinoculated after 23 days. Macroscopic lesions: testicle congested, ganglion hypertrophied. Histological lesions: vasodilatation, monocytic infiltration, and diffuse productive lesions. Ganglion: small parasites without endospores.

A718. Reinoculated with live material. Killed after 18-20 hours. Not vaccinated; reinoculated after 23 days. Macroscopic lesions: testicle congested. Histological lesions: discrete monocellular infiltration. No parasites observed.

A736. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated after 23 days. Macroscopic lesions: testicle hemorrhagic. Caseosis at the point of the inoculation. Nodule in lung. Histological lesions: necrotic and productive granuloma of the testicle, with very rare parasites of medium size with endospores.

F30. Reinoculated with dead material. Killed after 18-20 hours. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: discrete orchitis.

M12. Reinoculated with dead material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle slightly hypertrophied. Histological lesions: exudative nodular lesions.

F24. Reinoculated with live material. Killed after 18-20 hours. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: exudative orchiepididymitis with abundant eosinophiles. No parasites observed.

M11. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days.

Macroscopic lesions: testicle enlarged in size and congested; granulations in lungs. Histological lesions: productive orchitis and exudative and nodular interstitial epididymitis. Very rare parasites, small and with radiate formations.

F29. Reinoculated with dead material. Killed after 18-20 hours. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: orchitis and non-specific interstitial epididymitis. Spleen: productive and exudative nodular lesions with a few parasites.

M10. Reinoculated with dead material. Killed after 5 days. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: none.

F23. Reinoculated with live material. Killed after 18-20 hours. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested; ganglion hypertrophied. Histological lesions: testicle: interstitial exudative and productive lesions without parasites. Ganglion: follicular productive lesions, parasites with cystic endospores.

M9. Reinoculated with live material. Killed after 5 days. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: nodular exudative and productive epididymitis without parasites.

Group C. Guinea Pigs Infected Intraperitoneally and Reinoculated in the Testicle

A737. Reinoculated with dead material. Killed after 18-20 hours. Not vaccinated; reinoculated at the end of 20 days. Macroscopic lesions: testicle congested. Histological lesions: hemorrhagic and nodular focus with monocytic reaction, without parasites.

A773. Reinoculated with dead material. Killed after 5 days. Not vaccinated; reinoculated at the end of 20 days. Macroscopic lesions: testicle atrophied, caseosis at the point of the infecting injection, ganglion hypertrophied. Histological lesions: testicle: exudative-productive lesions with eosinophiles; parasites rare and small. Ganglion: granuloma with eosinophiles and abundant parasites.

A723. Reinoculated with live material. Killed after 18-20 hours. Not vaccinated; reinoculated at the end of 20 days. Macroscopic lesions: testicle congested. Histological lesions: edema and hemorrhage with exudative necrotic lesions without parasites.

A786. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated at the end of 20 days. Macroscopic lesions: testicle hemorrhagic, caseosis at the point of the infection; ganglion hypertrophied; nodules in lungs. Histological lesions: testicle: encysted granuloma with productive and exudative reaction, the former predominating. No parasites observed.

F96. Reinoculated with dead material. Killed after 18-20 hours. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle atrophied and contains pus. Ganglion hypertrophied and caseated. Granulations in the peritoneum and viscera. Histological lesions: testicle: exudative-productive granuloma with parasites in various evolutive states. Liver: granuloma without parasites.

F97. Reinoculated with dead material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size, with adhesions and caseum; abscesses in liver, spleen, and peritoneum. Histological lesions: specific interstitial orchitis with predominance of fibrous reaction. Epididymus: productive and exudative lesions containing parasites of medium size with radiate formations and others with polyhedral endospores.

F94. Reinoculated with live material. Killed after 18-20 hours. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle atrophied, with adhesions and caseum; granulations in peritoneum and viscera. Histological lesions: interstitial orchitis, exudative lesions in the epididymus with few parasites.

F95. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle atrophied and caseated. Histological lesions: specific interstitial orchitis with predominance of fibrous reaction. Epididymus: productive and exudative lesions containing parasites of medium size with radiate formations and others with polyhedral endospores.

F92. Reinoculated with dead material. Killed after 18-20 hours. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: discrete orchiepididymitis.

F93. Reinoculated with dead material. Killed after 5 days. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle congested. Histological lesions: exudative lesions. Spleen: granuloma without parasites.

F90. Reinoculated with live material. Killed after 18-20 hours. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle unchanged. Histological lesions:

necrotic exudative lesions and interstitial orchitis without parasites. Ganglion: few parasites, of medium size and without endospores.

F91. Reinoculated with live material. Killed after 5 days. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle atrophied and caseated. Histological lesions: exudative lesion with few parasites, one with polyhedral endospores.

Group D. Uninfected Guinea Pigs, Vaccinated in Various Ways, and Inoculated in the Testicle

R22. Inoculated with dead material. Killed after 18-20 hours. Vaccinated subcutaneously. Macroscopic lesions: testicle congested. Histological lesions: congestion, edema, and discrete monocytic infiltration.

R28. Inoculated with dead material. Killed after 5 days. Vaccinated subcutaneously. Macroscopic lesions: none. Histological lesions: none.

R21. Inoculated with live material. Killed after 18-20 hours. Vaccinated subcutaneously. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: edema with monocytic filtration without parasites.

R27. Inoculated with live material. Killed after 5 days. Vaccinated subcutaneously. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: nodular interstitial infiltration of monocytes and a great number of polynuclears; hemorrhagic foci with a great number of parasites in the infecting phase.

R24. Inoculated with dead material. Killed after 18-20 hours. Vaccinated intramuscularly. Macroscopic lesions: testicle congested. Histological lesions: congestion, edema, and discrete monocytic infiltration.

R30. Inoculated with dead material. Killed after 5 days. Vaccinated intramuscularly. Macroscopic lesions: none. Histological lesions: none.

R23. Inoculated with live material. Killed after 18-20 hours. Vaccinated intramuscularly. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: edema with monocytic infiltration without parasites.

R29. Inoculated with live material. Killed after 5 days. Vaccinated intramuscularly. Macroscopic lesions: testicle enlarged in volume and congested. Histological lesions: nodular interstitial infiltration of monocytes and a great number of polynuclears; hemorrhagic foci with a great number of parasites in the infecting stage. Young and adult parasites almost all infectious sporangia, only one with cystic spores.

R26. Inoculated with dead material. Killed after 18-20 hours. Vaccinated intraperitoneally. Macroscopic lesions: testicle congested. Histological lesions: zonal infiltration of polynuclears.

R25. Inoculated with live material. Killed after 18-20 hours. Vaccinated intraperitoneally. Macroscopic lesions: testicle unchanged. Histological lesions: testicle unchanged and without parasites.

R31. Inoculated with live material. Killed after 5 days. Vaccinated intraperitoneally. Macroscopic lesions: testicle enlarged in volume and congested. Histological lesions: nodular interstitial infiltration of monocytes and a great number of polynuclears; hemorrhagic foci with a great number parasites in the infecting phase.

Group E. Uninfected Guinea Pigs Inoculated Via the Testicle

L42. Inoculated with dead material. Killed after 18-20 hours. Macroscopic lesions: discrete congestion. Histological lesions: slight interstitial nodular reaction without parasites.

L43. Inoculated with dead material. Killed after 5 days. Macroscopic lesions: none. Histological lesions: none.

L40. Inoculated with live material. Killed after 18-20 hours. Macroscopic lesions: slight congestion. Histological lesions: congestion and discrete monocytic infiltration. One parasite of 10 microns.

L41. Inoculated with live material. Killed after 5 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: nodular productive lesions alternating with exudative ones; a great number of eosinophiles and of parasites in the infecting phase.

Discussion. The experimental study we have just described enables us to confirm the facts scientifically discovered by Posadas in 1892 and interpreted in the light of our present knowledge of allergy and immunity by the Italian school — Cavallero (10,11), Redaelli (12), and Redaelli and Ciferri (13).

C. Cavallero (1941) in his studies of allergy and immunity in mycoses called attention to the fact that in Gilchrist's disease (the blastomycosis of US authors) the primary cutaneous lesion is of the granulomatous type, while the secondary localizations via hematogen lead to rapid suppuration and then a tendency to spontaneous cure, which the author likens to Koch's phenomenon. Farina (cited by Cavallero) reproduced these facts experimentally and observed that the reinoculated germ disappeared after the sixth day. The reinoculated material sets up

a cellular reaction which passes through the following two phases: 1) a reactive phase of granulocytic character and 2) a resolving phase of granulomatous aspect with giant cells.

C. Cavellero (1943) encounters identity of coccidioidomycosis with tubercular infection, as had already been noted by Posadas and later in the US by C.E. Smith, Jacobson, Chipman, and Templeton. In coccidioidomycosis, besides the chronic form in which a granulomatous reaction similar to tuberculosis is observed, there exist the acute forms discovered by Gifford and Dickson (1936-1937) in which exudative-vascular phenomena predominate.

Redaelli and Ciferri (1942) described the reaction of the host to the material inoculated in experimental coccidioidomycosis in the following terms: a reaction of non-specific character is first produced, which tends to eliminate the foreign matter by digestion, and in which polynuclears, monocytic elements, and non-specific histocytic cells appear. There are elements of the germ that resist, "anamalize," multiply, and diffuse the infection. Then there begins a reaction of the conjunctive vascular tissue and particularly of the local reticuloendothelial system, which is characteristic of mycotic granuloma, the specificity of which is shown solely by the existence of a particular parasite, in this case C. immitis.

Hyperreceptivity and allergy put a particular stamp on the histogenic reactions of the animal infected. In hyperreceptive individuals, in a state of negative allergy, an exudative vascular reaction is observed followed by a series of regressive processes finally becoming necrotic and hemorrhagic, while in hyperresistant, hyperergic animals we observe the prevalence of hyperplastic reactive activity with the formation of granulomata with epithelial and giant cells and with a great quantity of plasmacellular and lymphoid elements arranged in the periphery, accompanied by a succession, varying in extent, of the fibroblastic series.

According to our studies intratesticular inoculation of material from cultures of C. immitis into a guinea pig not previously infected with coccidioidomycosis brings on in 18-20 hours a granulocytic and monocytic non-specific reaction which tends to eliminate the inoculated material as a foreign body; this is completely accomplished by the end of five days when material killed by heat has been inoculated. When on the other hand living material has been inoculated, the spores of the germ that have resisted this first non-specific defensive reaction evolve into the infectious phase, forming sporangia with a very fine peridial membrane which attain a diameter up to 83.5 µ and contain numerous very small polyhedral endospores (Posadas's granulose forms). The histogenic reaction is exudative-productive with abundant eosinophiles, as observed in

spontaneous infection in man (Wickoff and Usighi, cited by C.E. Smith, 1943).

Intratesticular reinoculation performed 26 to 43 hours after the first infecting inoculation gives rise after 18-20 hours to a reaction characterized by congestive-hemorrhagic phenomena and in some cases a necrotic exudative-productive reaction, which disappears by the end of five days when dead material has been inoculated but leads to a predominance of the productive reaction with formation of a granuloma when live material has been injected. This histogenic reaction, indicating a certain degree of immunity, is manifested by an almost complete disappearance of the parasites, and when these persist they appear in modified morphological and biological form. In fact, the parasites are medium to small, some 30 μ in diameter, without endospores and with the thick membrane frequently surrounded by an acidophilous areole or by radiate or claviform formations of the same staining properties.

Summary. Inoculation of material from cultures of Coccidioides immitis in the guinea pig in various ways leads to a state of hypersensitivity and partial immunity. The cutaneous hypersensitivity is quite evident on the seventh day and is accentuated on subsequent days.

Reinoculation with culture material killed by heat provokes at 18 to 20 hours an intense congestive-hemorrhagic reaction and histologically discernible exudative-necrotic phenomena which disappear toward the fifth day. Reinoculation with living material, on the other hand, induces productive phenomena which lead to the total or partial disappearance of the material injected, depending on the degree of immunity existing. This reaction, similar to Koch's phenomenon, also follows the biological law established by Levandowsky and Jadassohn in the case of leprosy.

In a guinea pig not previously infected, 18-20 hours after inoculation we observe a non-specific granulocytic and monocytic reaction which leads to total elimination of dead material by the fifth day. After inoculation with live material, on the other hand, the endospores that have resisted this first tissue reaction evolve toward the formation of primary infection sporangia, which are characterized by their enormous size (up to 83.5 μ in diameter), their fine membrane, and their content of granulose endospores without any apparent membrane of their own.

Vaccination with material killed by heat does not create cutaneous or tissual hypersensitivity, nor does it seem to modify the course and the reactivity of the organism in the face of a new inoculation with either living or dead material. Neither have we observed any variations due to the manner of the infecting inoculation [i.e. subcutaneous, intramuscular, intraperitoneal].

The parasite which develops within an organism in a state of allergy and partial immunity exhibits the following characteristics: its volume is generally less, the production of endospores is notably restricted, and when such production does take place it leads to the production of cystic endospores (Posadas's vegetative or cystic phase). The peridial membrane is always thick and frequently surrounded with an acidophilous areole or with radiate or claviform formations of the same staining properties.

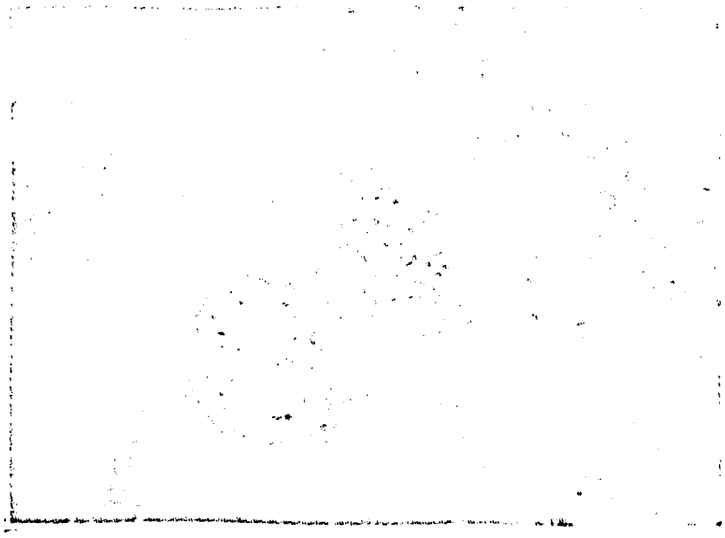

Figure 1. Sporangia with granulose endospores.

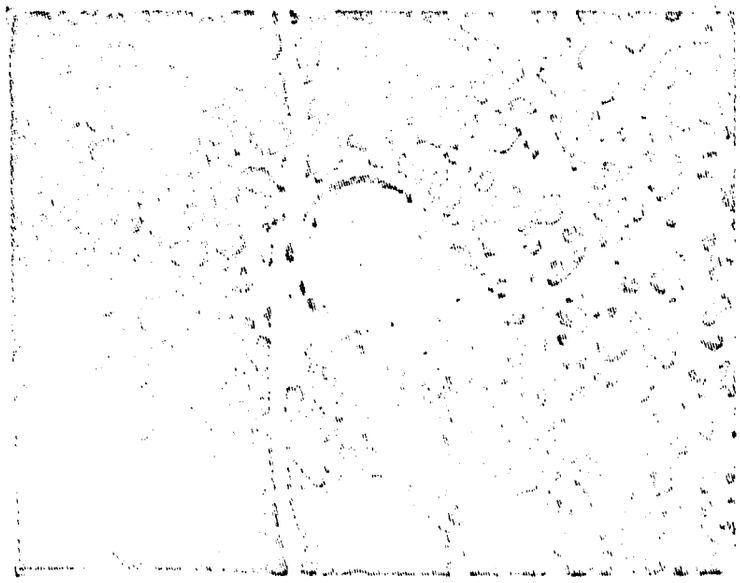

Figure 2. Sporangium with granulose endospores, in dehiscence.

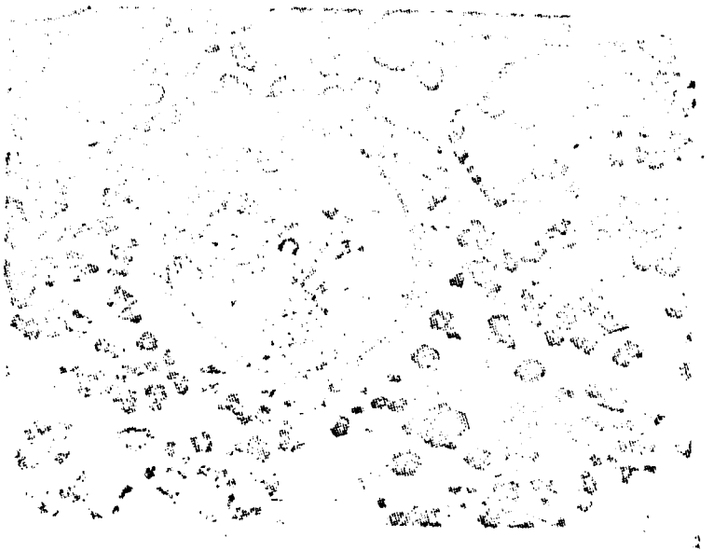

Figure 3. Sporangium with cystic endospores.

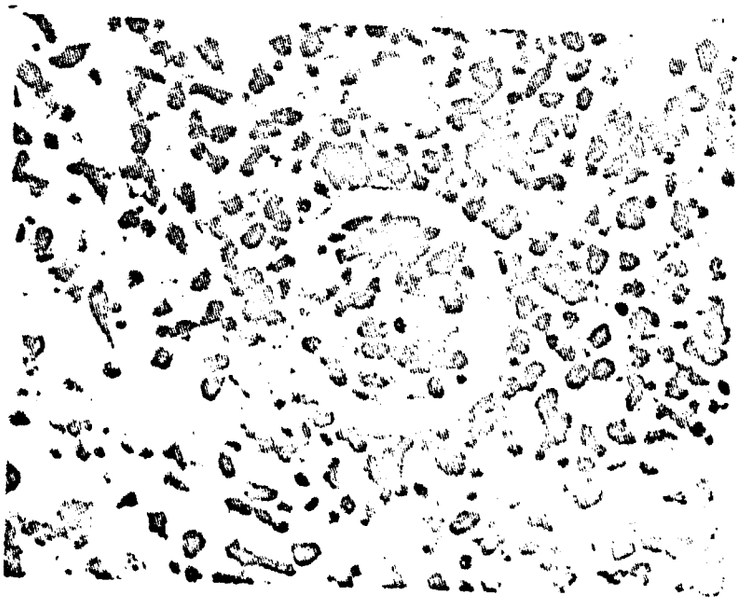

Figure 4. Empty sporangium invaded by leucocytes.

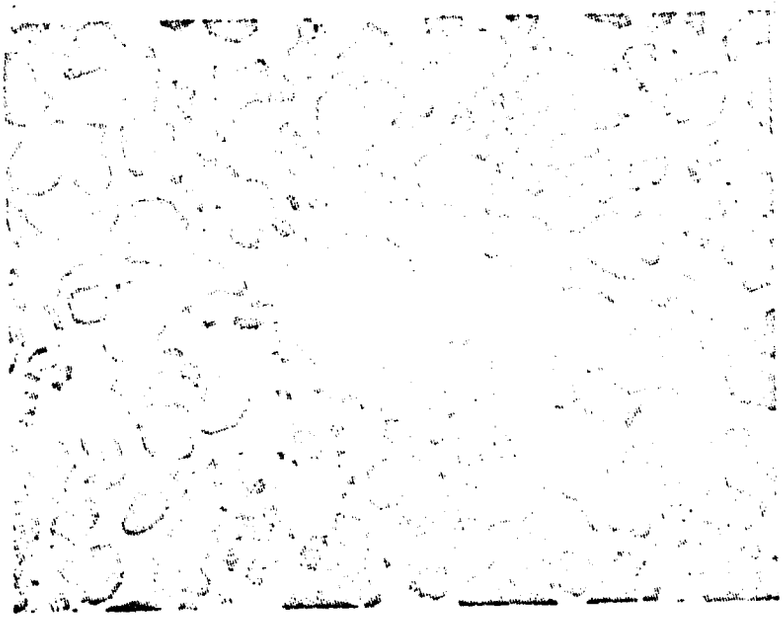

Figure 5. Cystic parasite with radiate formations.

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